Acta Crystallographica Section D
Biological Crystallography
ISSN 0907-4449

Crystallization and preliminary crystallographic studies of human TGF-β type II receptor ligand-binding domain

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Received 11 January 2002
Accepted 22 April 2002

Three constructs (residues 15–136, 22–136 and 27–136) of the truncated extracellular domain of human transforming growth factor β type II receptor (TBRII) were overexpressed in *Escherichia coli*. The constructs are referred to as TBRII(15–136), TBRII(22–136) and TBRII(27–136). The refolded receptors were purified using a combination of ion-exchange and size-exclusion chromatography. The purified receptors have an apparent molecular weight of 14 kDa as judged by size-exclusion chromatography. In the crystallization trials, TBRII(15–136) and TBRII(22–136) formed mostly crystal-like spheres but failed to produce data-quality crystals. TBRII(27–136) yielded large single crystals from hanging drops using the vapor-diffusion procedure with PEG 2000 or 4000 at pH 5.0. The crystals diffracted to 1.05 Å [using the X9B beamline operated at $\lambda = 1.0092$ Å of the National Synchrotron Light Source (NSLS) at the Brookhaven National Laboratory] and belong to space group $P2_12_12_1$, with unit-cell parameters $a = 35.5$, $b = 40.7$, $c = 76.2$ Å. There was one molecule in the asymmetric unit, which corresponds to a solvent content of 42.1%.

1. Introduction

Transforming growth factor β (TGF-β) is found in most eukaryotic organisms, including *Clostridium elegans*, *Drosophila*, *Xenopus*, mice and humans. It is expressed by virtually every cell type in most stages of development and is involved in a wide range of biological functions including development, epithelial cell growth, carcinogenesis and immune regulation (Massague, 1998; Letterio & Roberts, 1998). Structurally, TGF-β belongs to a cystine-knot superfamily of homologous growth factors, including activin and bone morphogenic protein (Daopin *et al.*, 1992; Schlunegger & Grutter, 1992; Sun & Davies, 1995).

TGF-β binds to the cell-surface receptor TBRII, forming a heterodimer capable of recruiting and activating the type I receptor (TBRI). In the absence of TBRII, TGF-β has no affinity for TBRI. Upon complex formation, the constitutively active serine/threonine kinase in the cytoplasmic domain of TBRII phosphorylates the kinase domain of TBRI, thereby initiating a signaling cascade through the SMAD molecules (SMAD refers to homologous genes originally identified as Sma in *C. elegans* and MAD in *Drosophila*; Massague, 1998; Heldin *et al.*, 1997).

TBRII is a type I transmembrane glycoprotein containing a 136-residue TGF-β-binding domain (Lin *et al.*, 1992). The structures of two other members of the TGF-β receptor superfamily have been solved. These are the soluble extracellular domain of the mouse type II activin receptor (Greenwald *et al.*, 1999) and a complex between the bone morphogenetic protein 2 and its type IA receptor extracellular domain (Kirsch *et al.*, 2000). Both receptor structures have a three-finger toxin fold, structurally similar to some snake-venom neurotoxins.

2. Materials and methods

2.1. Cloning, overexpression and purification

Three constructs of TBRII were made in an attempt to crystallize the receptor and were named TBRII(15–136), TBRII(22–136) and TBRII(27–136) according to the residues they include (Boesen *et al.*, 2000). In brief, the extracellular TGF-β-binding domain of human TBRII with the mutation K97T was cloned into a pET30a vector (Novagen, Madison, Wisconsin, USA) [in Boesen *et al.* (2000) the K97T mutation was incorrectly identified as K98T]. *E. coli* BL21(DE3) cells containing the TBRII plasmid were grown in 10 l of Super Broth in a Bioflo 3000 bioreactor (New Brunswick Scientific, Edison, New Jersey, USA). Upon harvest, cells were lysed using a microfluidizer (Microfluidics Corporation, Newton, Massachusetts, USA) and the inclusion bodies were washed with a buffer containing 1.5 *M* urea. For refolding, inclusion bodies were dissolved in 6 *N* guanidine hydrochloride and then injected into a solution

containing 0.5 *M* arginine, 5 m*M* cysteamine and 0.5 m*M* cystamine. The refolded receptor was collected on a Source 15Q column (Amersham Pharmacia Biotech, Piscataway, New Jersey, USA) with a gradient elution of 0–0.75 *M* NaCl in 25 m*M* Tris pH 8.0. The eluted receptor was concentrated to 1.0 ml, loaded onto a Mono P HR 5/20 column (Amersham Pharmacia Biotech, Piscataway, New Jersey, USA) and eluted with a 0–0.5 *M* NaCl linear gradient in 50 m*M* HEPES pH 7.0. The Mono P peak was further purified using a Superdex 200 HR 10/30 column (Amersham Pharmacia Biotech, Piscataway, New Jersey, USA) at a 0.5 ml min^{-1} flow rate in 50 m*M* NaCl, 50 m*M* Tris pH 8.0. All chromatography was carried out at 277 K using an ÄKTA purifier (Amersham Pharmacia Biotech, Piscataway, New Jersey, USA).

2.2. Crystallization and X-ray data collection

For crystallization experiments, each of the three variants of the receptor was dialyzed against water and concentrated to 20 mg ml^{-1} using a Speedvac concentrator. Crystallization screening experiments were carried out using the Hampton Crystal Screen kit I (Hampton Research, Laguna Hills, California, USA) by vapor diffusion in hanging drops at room temperature. After a brief soaking in precipitant solutions containing 20% glycerol, the receptor crystals were flash-frozen at 100 K. The highest resolution X-ray diffraction data was collected from single crystals using an ADSC Quantum IV CCD detector at a crystal-to-detector distance of 70 or 120 mm at the X9B beamline of the National Synchrotron Light Source (NSLS) at Brookhaven National Laboratory operated at λ = 1.0092 Å and were processed with *HKL*2000 (Otwinowski & Minor, 1997). 360 frames were collected with a 0.5° oscillation and 15 s exposure per frame at a crystal-to-detector distance of 70 mm. 180 frames were collected with a 1° oscillation and 5 s exposure per frame at a crystal-to-detector distance of 120 mm.

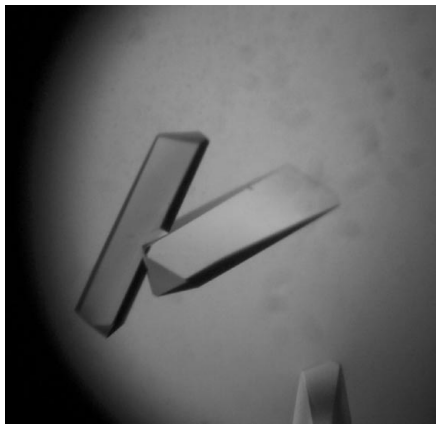

Figure 1
Crystals of the truncated extracellular domain of TBRII(27–136) were grown to approximate dimensions of 0.2 × 0.4 × 1.0 mm.

3. Results and discussion

The purification of TBRII(15–136) and TBRII(22–136) has been described previously (Boesen *et al.*, 2000). The purification of TBRII(27–136), however, was slightly different. Refolded TBRII(27–136) was first loaded onto a Source 15Q column and eluted as three individual peaks. The first two peaks from the Source 15Q column (fractions 24–32 ml) were concentrated and then resolved on the Mono P column. The first peak from the Mono P column (fractions 12–15 ml) ran as a single sharp peak on the Superdex 200 HR 10/30 column. The elution profile of the receptor corresponds to an apparent molecular weight of 14 kDa.

About 15% of Crystal Screen kit I conditions testing TBRII(15–136) resulted in spherulites (crystal-like spheres), often in the presence of precipitation, in 12–36 h. In one condition [15% PEG 8000, 0.2 *M* $Ca(OAc)_2$, 0.1 *M* HEPES pH 7.0], one to three crystals too small for X-ray diffraction experiments appeared 24–48 h after the appearance of spherulites with precipitation in the same drop. About 10% of Crystal Screen kit I conditions testing TBRII(22–136) resulted in spherulites, often in the presence of precipitation, in 12–36 h. No conditions resulted in crystals.

Approximately 20% of Crystal Screen kit I conditions testing TBRII(27–136) resulted in spherulites, often in the absence of precipitation, in 12–36 h. One condition in the initial crystallization screens yielded thin plate-shaped crystals which appeared in 12 h in 30% PEG 1500. The addition of a pH buffer reproduced these thin plate-shaped crystals at neutral pH. In the final conditions, single crystals were obtained from 1–3 µl drops containing a 1:1 mixture of protein and a well solution composed of 30% PEG 2000 or 25% PEG 4000 and 0.1 *M* sodium citrate at pH 5.0. Crystals appeared after 7 d and grew to average dimensions of 0.2 × 0.4 × 1.0 mm in approximately one week (Fig. 1).

The most likely explanation for the different crystallization behavior of the three constructs is that only TBRII(27–136) was folded correctly. Refolding from two separate inclusion-body preparations of TBRII(22–136) resulted in variable yields of soluble protein which ran as a single broad peak on a size-exclusion column, indicating that it may not be correctly folded. Each batch of TBRII(27–136) refolding from inclusion bodies, however, gave a high yield of soluble protein. Although refolding from one inclusion-body preparation of TBRII(27–136) ran as a single broad peak, refolding from a second inclusion-body preparation gave a single sharp peak on a size-exclusion column. The TBRII(15–136) construct could only be expressed in *E. coli*

Table 1
Data-collection statistics.

Values in parentheses are for the highest resolution shell (1.09–1.05 Å).

Resolution limit (Å)	1.05
Unique reflections	45991 (2277)
Redundancy	8.5 (3.9)
Completeness (%)	87.9 (44.3)
R_{sym}† (%)	3.5 (48.8)
$\langle I/\sigma(I)\rangle$	56.5 (2.8)

† $R_{sym} = 100\sum|I_h - \langle I_h\rangle|/\sum I_h$, where $\langle I_h\rangle$ is the mean intensity of multiple measurements of symmetry-equivalent reflections.

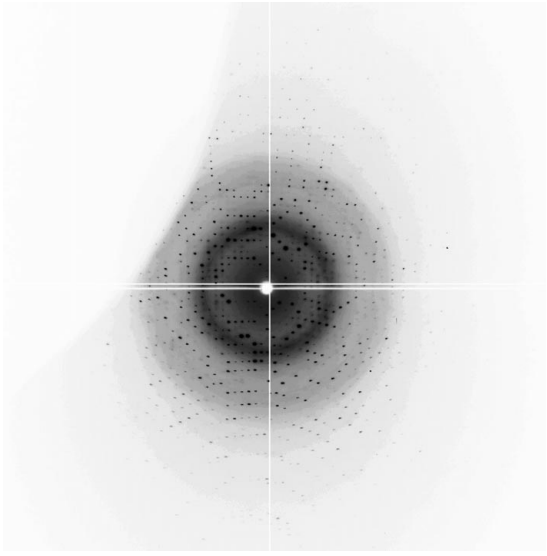

Figure 2
A typical diffraction pattern from a crystal of the truncated extracellular domain of TBRII(27–136) at 100 K from a 0.5° oscillation and 15 s exposure on a ADSC Quantum IV CCD detector at a crystal-to-detector distance of 70 mm using the X9B beamline operated at λ = 1.0092 Å of the National Synchrotron Light Source (NSLS) at the Brookhaven National Laboratory. The diffraction limit to the edge of the image was 1.0 Å.

as a soluble fusion protein with glutathione *S*-transferase and may not have been properly folded. Since TBRII(27–136) is the shortest construct that still contains all 12 cysteine residues, it is possible that the five extra residues in TBRII(22–136) inhibit refolding.

The crystals had a mosaicity value of 0.35° and diffracted to 1.05 Å. Fig. 2 shows a typical diffraction pattern and Table 1 contains the data-set statistics. The crystal belongs to space group $P2_12_12_1$, with unit-cell parameters $a = 35.5$, $b = 40.7$, $c = 76.2$ Å. Assuming one receptor molecule per asymmetric unit cell, the calculated V_M was 2.14 Å^3 Da^{-1} (Matthews, 1968), corresponding to a solvent content of 42.1%.

We wish to thank Agniswamy Johnson and Michael Kattah for their assistance in synchrotron data collection.

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